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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENELIGLIPTIN AND DAPAGLIFLOZIN API IN MARKETED FORMULATION

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Keywords:

RP-HPLC, Teneligliptin hydrobromide hydrate, Dapagliflozin propanediol monohydrate, Method Development, Validation

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ABSTRACT: A simple, novel, precise, rapid, accurate, specific reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of Teneligliptin hydrobromide hydrate and Dapagliflozin propanediol monohydrate API in marketed formulation was developed and validated. The separation was carried out on a Prontosil C18 (250 x 4.6 mm, 5 m) column using an acetonitrile: 3.5 pH Potassium dihydrogen phosphate buffer (60:40 v/v) mobile phase. The flow rate was 1 mL/min, and the UV detector was set to detect at 227 nm. Teneligliptin Hydrobromide Hydrate retained 2.37 min, while Dapagliflozin Propanediol Monohydrate retained 3.61 min. Teneligliptin hydrobromide hydrate revealed a linear response in the concentration range of 10-30ppm. In the concentration range of 5-15ppm, dapagliflozin propanediol monohydrate demonstrated a linear response. Teneligliptin hydrobromide hydrate and Dapagliflozin propanediol monohydrate had correlation coefficients ('R^{2}' value) of 0.999 and 0.992, respectively. Various validation parameters were used to validate the analysis results. Teneligliptin hydrobromide hydrate and Dapagliflozin propanediol monohydrate percentage recoveries vary from 98% to 101%. Both precision and robustness study found to have % RSD values of less than 2. Thus, the suggested method may be successfully used in routine analysis for the method development and validation of Teneligliptin Hydrobromide Hydrate and dapagliflozin propanediol monohydrate.

INTRODUCTION: Teneligliptin hydrobromide hydrate (TEN) has the following chemical name (2S, 4S) pyrrolidin-2-yl) (1,3-thiazolidin-3-yl) (4-[4-(3-methyl-1phenyl-1Hpyrazol-5-yl) piperazin-1-yl] pyrrolidine-2-yl hemi methadone **Fig. 1** [1, 2]. A dipeptidyl peptidase is Penta hydrobromide hydrateinhibitor [1]. Teneligliptin tends to slow the inactivation of incretin hormones in type 2 diabetes patients, enhancing bloodstream concentrations while decreasing fasting and postprandial glucose concentration levels in a glucose-dependent manner [3].

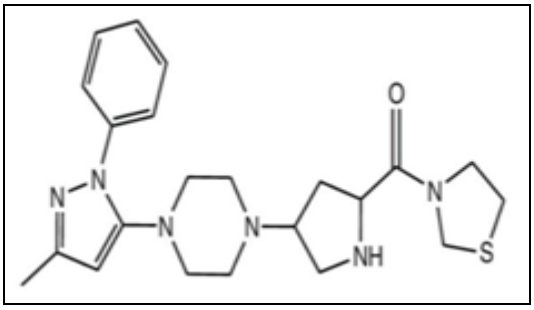

FIG. 1: CHEMICAL STRUCTURE OF TENELIGLIPTIN

Dapagliflozin is also known as (1s) -1, 5-anhydro-1-C-[4 – chloro – 3 - [(4 - ethoxyphenyl) methyl] phenyl] - 1, 5 – anhydro – 1 – C - [4 – chloro – 3 - [(4 – ethoxypheny – D - glucitol **Fig. 2** [1-2], Dapagliflozin inhibits renal glucose reabsorption via the solid- glucose cotransporter (SGLT), allowing for an insulin-independent technique of Controlling blood glucose levels in type 2 diabetes patients is essential [3].

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Dapagliflozin is a first-generation selective SGLT inhibitor that is anticipated to be 100-fold more selective for SGLT2 than SGLT1 [3]. Teneligliptin and Dapagliflozin are drugs that are used to improve glycemic control in people with type 2 diabetes [1, 3].

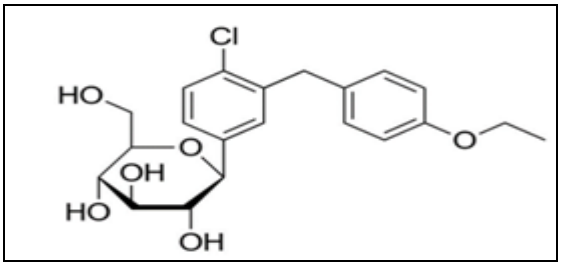

FIG. 2: CHEMICAL STRUCTURE OF DAPAGLIFLOZIN

Several spectroscopic RP-HPLC techniques for estimating Teneligliptin and Dapagliflozin alone and in combination with other drugs have been published [3, 4, 7, 8]. As a result, it was thought desirable todevelop an accurate, precise, and cost-effective fast RP-HPLC method for the simultaneous measurement of Teneligliptin and Dapagliflozin in tablet dose form.

MATERIALS AND METHOD:

Instrumentation: Chromatography was carried out on a Shimadzu prominence - i 2030 system with data processing software from lab solution. The separation and quantification were performed on a Prontosil C 18 column (250×4.6 nm,5m).

Chemicals and Reagents:

Active Pharmaceuticaling Redients used: Teneligliptin Hydrobromide Hydrate Purity of API is (99%) and Dapagliflozin Propanediol Monohydrate purity of API (99%).

Pharmaceutical Formulation: Tablets of Teneligliptin Hydrobromide Hydrate and Dapagliflozin Propanediol Monohydrate with strength 10 mgand 20mg Brand name as ZITA-D manufactured by Glenmark were used. These tablets were purchased from local pharmacy.

Reagentsand Chemicals used: Acetonitrile (Merck) and Milli-Qwater (HPLC Grade), Potassium dihydrogen Phosphate, Orthophosphoric Acid were used for preparing mobile phase.

Selection of Wavelength: Each solution of selected API was scanned using a twin beam UV visible spectrophotometer from 200 nm to 400 nm, yielding overlapping spectra. The wavelength chosen was 227 nm, which corresponds to the isosbestic point. **Fig. 3** depicts the superimposed spectra of Teneligliptin and Dapagliflozin.

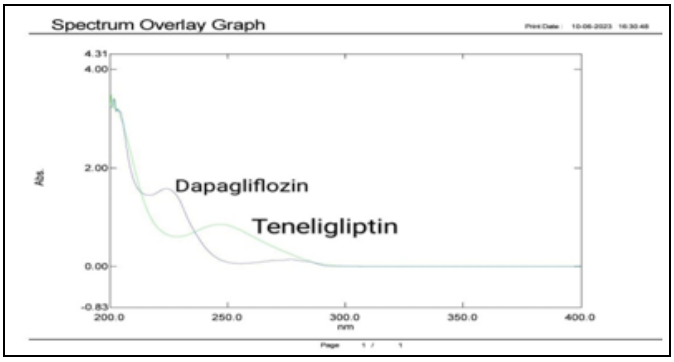

FIG. 3: UV SPECTRUM OF DAPAGLIFLOZIN AND TENELIGLIPTIN

Chromatographic Condition: A Prontosil C18 (250 x 4.6 mm, 5 m) column was used to develop the procedure. Acetonitrile: 3.5 pH potassium dihydrogen phosphate buffer (60:40) was used as the mobile phase. To identify the detection wavelength of 227 nm, a typical drug solution was scanned with a spectrometer over a wide range of wavelengths 200-400 nm. The pump's flow rate was set to 1 mL/min, and the capacity was set to 10µL. The temperature in the column was set to 30°C.

Preparation of Mobile Phase: 60 volumes of HPLC grade acetonitrile and 40 volumes of Buffer was used as mobile phase.

Preparation of Buffer pH 3.5: Dissolve 2.7 g of Potassium Dihydrogen Phosphate in 900ml water. Adjust the Ph 3.5 with Phosphoric Acid. Diluted to 1000 mL with water [9].

Preparation of Diluent: Water: Acetonitrile (60:40) was chosen as a diluent based on the solubility of the medication.

Preparation of Standard Solution: 10mg Teneligliptin and 10mg Dapagliflozin working standard were accurately weighedtransferredinto10 mL volumetric flask respectively. About 4ml diluent was added, sonicated to dissolve and diluted to 10mL using diluent. Accurately 2mL of Teneligliptin and 1mL of dapagliflozin both the solutions were transferred in to 10mL volume tricflask, Volume was make up with diluents to get

20ppm of Teneligliptin hydrobromidehydrate and 10ppm of Dapagliflozin propanediol monohydrate.

Preparation of Sample Solution: Ten tablets were crushed to make a fine powder. The tablet powder equivalent to 20 mg of Teneligliptin and 10 mg of Dapagliflozin was transferred to a 100 mL volumetric flask and dissolved in diluent for 30 minutes while the flask was ultrasonicated. Finally, diluent was used to get the volume up to the required level. This solution was further diluted by taking 1 mL from the above solution and diluting it to 10 mL with diluent.

Method Development: Chromatographic separation was accomplished using a Prontosil C18 (250 x 4.6 mm, 5 m) column with acetonitrile and buffer in the ratio of (60:40) as the mobile phase at a flow rate of 1 mL/min and column temperature of 30°C, with detection at 227 nm. The optimized approach resulted in an elution time of 2.37 min for Teneligliptin and 3.61 min for Dapagliflozin. The overall running time was 8 min. **Fig. 2** and **3** exhibit chromatograms of Teneligliptin and Dapagliflozin standard and sample, respectively.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR TENELIGLIPTIN AND DAPAGLIFLOZIN

Parameters	Optimized conditions
Pump mode	Gradient
Column	Prontosil C18 (250 x 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: Buffer (60:40)
Flow rate	1mL/min
Column temperature	30°C
Injection Volume	10 µL
Detection wavelength	227 nm
Retention time	2.37 min and 3.61 min respectively

RESULT AND DISCUSSION: The developed RP-HPLC method for Teneligliptin and Dapagliflozin was validated as per ICH guidelines [5].

Specificity: Specificity is defined as the capacity to evaluate an analyte definitively in the presence of predicted components [5]. The specificity of the approach was determined by watching and comparing the test result obtained for the sample solution with the standard result obtained for a pure drug, as shown in **Fig. 4, 5, 6.**

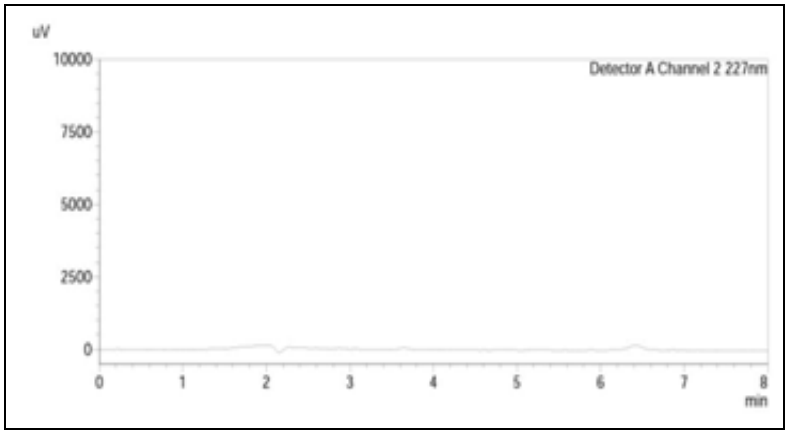

FIG. 4: CHROMATOGRAM OF BLANK SOLUTION

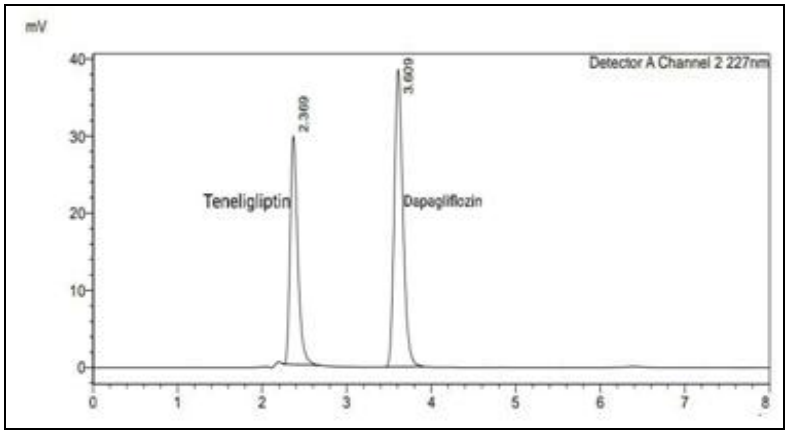

FIG. 5: CHROMATOGRAM OF STANDARD SOLUTION OF TENELIGLIPTIN AND DAPAGLIFLOZIN

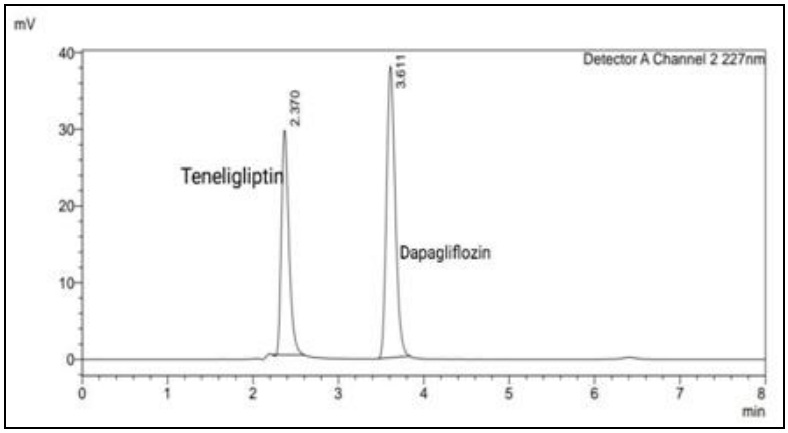

FIG. 6: CHROMATOGRAM OF SAMPLE SOLUTION OF TENELIGLIPTIN AND DAPAGLIFLOZIN

System Suitability: In order to confirm the optimal settings, the system suitability parameter was studied. A system appropriateness test was performed on the chromatograms in accordance with USP requirements [5]. The following parameters were assessed: retention time, tailing factor, theoretical plate, and resolution. The results are summarized in **Table 2.**

TABLE 2: SYSTEM SUITABILITY PARAMETER RESULTS

Parameter	Teneligliptin	Dapagliflozin
Retention time (min)	2.37	3.61
Tailing factor (Less than 2)	1.33	1.22
Theoretical plate (More than 2000)	3173	5464
Resolution	-	6.856

Linearity: The concentrations of teneligliptin and dapagliflozin were utilized to produce the standard curve. The method's linearity was examined using linear regression analysis [5]. The linearity graph was generated by displaying the drug concentration on the X-axis and the corresponding peak area on the Y-axis, as illustrated in **Fig. 7** and **8**. **Table 3** summaries the linearity statistics.

TABLE 3: LINEARITY DATA OF TENELIGLIPTIN AND DAPAGLIFLOZIN

Concentration of Teneligliptin (ppm)	Peak Area of Teneligliptin	Concentration of Dapagliflozin (ppm)	Peak Area of Dapagliflozin
10	110395	5	134867
15	160065	7.5	170411
20	206440	10	221310
25	312097	12.5	281825
30	312097	15	335539
Correlation Coefficient (R^2)	0.9993	Correlation Coefficient (R^2)	0.9924

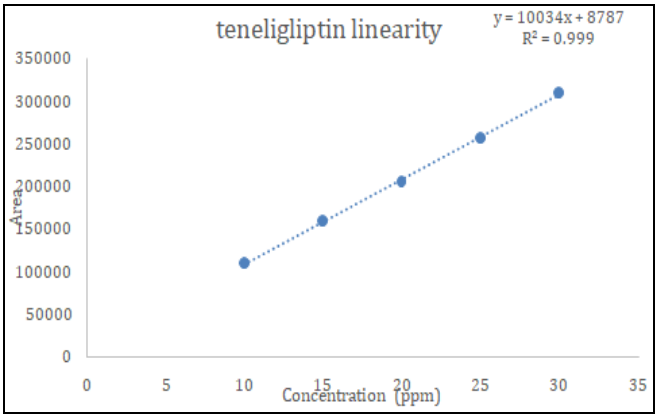

FIG. 7: CALIBRATION CURVE OF TENELIGLIPTIN HYDROBROMIDE HYDRATE

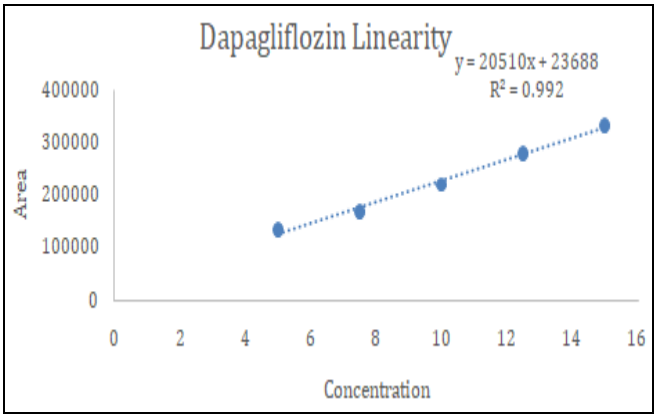

FIG. 8: CALIBRATION CURVE OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE

Precision: An analytical procedure's precision defines the level of agreement between a number of measurements taken from multiple sampling if the same sample is used under identical conditions [5]. The precision of an analytical technique expresses the degree of agreement between a set of measurements acquired from multiple samplings of the same sample under prescribed conditions. To achieve system precision (20 ppm), six replicate injections of Teneligliptin standard solution were employed. The average, standard deviation (SD), and percentage RSD of area were calculated and reported for six replicate injections. Furthermore, triplicate injections of Dapagliflozin (10 ppm) and Teneligliptin standard and sample solutions (20 ppm) were done. Its assay, average, standard deviation (SD), and percent RSD were computed and presented as follows. **Table 4** highlights the findings of the system precision and technique precision tests.

TABLE 4: SYSTEM PRECISION & METHOD PRECISION RESULT

Injection	System Precision Area of Standard		Method Precision % Assay	
	Teneligliptin	Dapagliflozin	Teneligliptin	Dapagliflozin
1	183984	274764	99.4	100.3
2	180267	266642	100.4	101.3
3	178611	269096	100.6	100.7
4	179836	274750	98.2	98.1
5	180415	273658	99.9	100.34
6	180492	274750	100	98.23
Mean	180492	272277	100	100
SD	1824.7985	3526.9185	0.8666	1.338
%RSD	1.01	1.30	0.87	1.34

Accuracy /Recovery Studies: Recovery tests were carried out to investigate the suggested method's accuracy and reproducibility [5]. The standard medication was added to a predetermined amount of pre-analyzed sample at 50%, 100%, and 150% concentrations. Each level was repeated three times. **Table 5** displays the Teneligliptin and Dapagliflozin content discovered utilizing the proposed approach. Teneligliptin and Dapagliflozin had mean recoveries of 99.89% and 99.46%, respectively.

TABLE 5: ACCURACY DATA OF TENELIGLIPTIN AND DAPAGLIFLOZIN

Pre-analyzed sample	Level	Amount Added (PPM)	Amount Recovered (PPM)	% Recovery	Mean of % recovery
Teneligliptin	50%	8	8.03	100.38	
	100%	10	10.01	100.15	99.89
	150%	12	11.9	99.16	
Dapagliflozin	50%	16	15.98	99.89	
	100%	20	19.7	98.5	99.46
	150%	24	24.02	100	

Assay of Marketed Formulation: Ten tablets were weighed and finely pulverized. Transfer tablet powder equivalent to 20 mg of Teneligliptin and 10 mg of Dapagliflozin into a 100 mL volumetric flask, add 60 mL diluent, sonicate for 30 minutes, and make up the volume to the mark. This solution was diluted further by placing 1 mL from above solution in a 10 mL volumetric flask and filling the remaining capacity with diluent to achieve 20 ppm of Teneligliptin acid and 10 ppm of Dapagliflozin.

TABLE 6: ANALYSIS OF THE FORMULATION

Tablet	Drug	% Assay
Zita-D Tablet	Teneligliptin	100.12
	Dapagliflozin	100.6

Robustness: Robustness is a measure of its ability to remain unaffected by minor changes in the chromatographic technique parameters and indicates its dependability. The ability to stay unaffected by tiny changes in the chromatographic technique parameters implies robustness, which

indicates dependability. The retention time of Teneligliptin and Dapagliflozin was measured after making slight purposeful modifications in the chromatographic conditions at three different levels. Flow rate, column temperature and wavelength were chosen as parameters. There were no deliberate changes in the chromatogram, demonstrating the robustness of the developed RP-HPLC method, **Table 7** describes the outcome.

TABLE 7: RESULT OF ROBUSTNESS STUDY

Parameter	Level	Teneligliptin			Dapagliflozin		
		Area	Number of Theoretical Plates	Peak Tailing	Area	Number of Theoretical Plates	Peak Tailing
Flow Rate	0.9 mL/min	206621	3141	1.45	306012	5576	1.25
	1mL/min	206580	2989	1.39	221550	5288	1.23
	1.1 mL/min	185179	2916	1.38	247182	5166	1.23
Temperature	29°C	185889	3058	1.37	280033	5304	1.24
	30°C	206580	2989	1.39	221550	5288	1.23
	31°C	187549	3074	1.39	273029	5525	1.24
Wavelength	226 nm	188324	3036	1.39	284353	5358	1.23
	227 nm	206580	2989	1.39	221550	5288	1.23
	228 nm	183690	2996	1.36	269115	5293	1.23

CONCLUSION: According to the findings, the RP-HPLC method for simultaneous estimates of Teneligliptin and Dapagliflozin pharmaceutical formulation was successfully established. Both medications have a high resolution with short analysis time of 8-min. The developed HPLC method was found to be simple, accurate, linear, precise, and robust. It is capable of determining the individual and concurrent concentrations of Teneligliptin and Dapagliflozin in pharmaceutical medication items and substances. It can be used to determine the assay of medicinal products, mix consistency, and content uniformity. The developed approach was validated in compliance with ICH recommendations, and the findings were equivalent.

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CONFLICTS OF INTEREST: Nil

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